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VOLUME XL NUMBER 5

BOTANICAL GAZETTE

NOVEMBER, 1905

STUDIES OF IRRITABILITY IN ALGAE.

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(WITH TWENTY-SEVEN FIGURES)

INTRODUCTION.

IN a recent book on the physiology of plants (**13**) the statement is made that "when zoospores and other motile and floating spores of sessile plants come to rest, attaching themselves to the substratum, the attachment is effected through means not yet wholly clear. . . It remains for experiment actually to show that the increased rate and the changed direction of growth in such cases among plants are due to contact, though it seems to be the case in animals" (**10**).

It was with a view to adding somewhat to our knowledge in this direction that the studies here reported were undertaken, first with fresh-water algae, and subsequently with marine. We worked together on the fresh-water algae; one of us worked alone on the sea-weeds;[1] we have gone over all the results together.

For the sake of clearness, we may briefly describe the series of events, a small part of which we wish to report in some detail. Zoospores of Oedogonium, Vaucheria, and other fresh-water algae, after escaping from the cells in which they form, may swim about for a considerable length of time. If they are in the light, the direction of their locomotion is influenced by the direction and intensity of the light which falls upon them. This has long been known, though even now far from perfectly understood (7). When the zoospores come to rest, they surround themselves with a cellulose wall. If they come to rest in contact with a solid object, their form changes as well; the part against the solid flattens and adheres to it. Thus a holdfast is formed. The young plant, now sessile, may pass rapidly by successive periods of cell-division and of growth through the subsequent stages of its development. The activities of zoospores, then, are greatly influenced at different times by various agents; the direction of their locomotion is determined by the intensity and direction of the light falling upon them, the formation of cell-wall immediately follows their coming to rest; the growth of the foot or holdfast is proportioned in form and extent to the roughness of the surface with which the zoospore is in contact; and the direction of the first cell-division is probably determined mainly by the light. These last three points we shall discuss in detail.

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[1] I take this opportunity to express my appreciation of the opportunity of studying, during October and November 1904, at one of the tables maintained at the Zoological Station in Naples by the Carnegie Institution, Washington. I wish also heartily to thank the officers of the Zoological Station for the courtesy which they showed me.—G. J. P.

Turning for the moment to the behavior of the non-motile spores of marine algae, such as those of Fucus, Cystoseira, Dictyopteris (Halyseris), we see nearly the same phenomena exhibited. There is no independent locomotion, and hence the transport of the spores from their points of origin to where they are to germinate is not directed by the influence of light upon them, but by water currents. The formation of the cellulose wall, the growth of the foot, and the direction of the first cell-division (20) are, however, determined by the same influences as those controlling the similar phenomena among fresh-water algae. These we hope the following pages will make clear. We shall report first upon the fresh water algae, as it was upon these that we began our work.

I. FRESH-WATER ALGAE.

MATERIAL AND METHOD.

Vigorous plants of Oedogonium, somewhat crowded by diatoms and other low forms, were found in watering-troughs for horses in paddock or on pasture in the vicinity of Stanford University, California. Many of the plants were growing attached to the sides of the troughs, submersed but near the surface of the water, in some cases fully exposed to the light, in others partly shaded by trees. Other Oedogonium filaments were found attached to dead leaves floating near the surface of the water in the troughs. This water was quiet and fairly clear. The level of the water, tending to be lowered by evaporation or by the draughts of the few horses using the troughs, was automatically maintained by ball-valves, similar to those used in houses. A few other plants, apparently of the same species, were found in a quiet pool in the San Francisquito Creek, growing on stones in comparatively clear water.

The material was brought fresh into the laboratory from time to time from mid-September till mid-November, and again from early April till late in May. Since the material did not fruit in our cultures, and we found none fruiting out of doors, it was impossible to determine the species, though we tried to have this done for us. We regret this lack of definiteness in our work.

METHOD OF MANIPULATION.

Plants brought into the laboratory were placed in an abundance of tap-water in glass dishes covered with a glass plate or loose cap to prevent excessive evaporation and to exclude dust. Small Stender dishes were largely used, since these could easily be placed under the microscope, thereby avoiding such disturbance of the material as transfer from one dish to another would entail.

Fortunately for us the water from the tap was from the same source that supplied the horse-troughs, and the creek contains mainly the overflow from the artificial lake which is the general water-supply for the region. When, therefore, our plants were brought in and put into tap-water in the laboratory, they were put into water of the same composition and approximately of the same temperature and degree of aeration as the water of the troughs and the pool from which they had been removed. The behavior of our plants was immediately affected only by those factors changed by the transfer to the laboratory, not by the water, though it goes without saying that the temperatures of the cultures in the laboratory never fell quite so low at night during the winter and spring as that of the water outside in troughs and pools.

Cultures were also made in Knop's solution,[2] 1 per cent. and 0.5 per cent. In certain cases we added 10 per cent. gelatine or 0.25 per cent. agar-agar to the Knop's solution, in order to obtain a solid culture medium. These solid cultures were made in Petri dishes. Although such dishes have the same advantage as Stender dishes in that they can be put under the microscope and the cultures studied without disturbance, they cannot safely be employed in an ordinary laboratory for more than very brief periods at a time. The air of the ordinary laboratory is excessively dry (**2**). In consequence, there will be a quite too rapid and altogether excessive concentration of the culture medium in Petri dishes, even if covered, in all cases in which experiments last for more than a few days. In our climate, the air in the laboratory in which we worked is more humid during the winter (the rainy season) than in most laboratories. We therefore ventured to continue these cultures for some time. In summer and autumn here, and at all seasons in most laboratories elsewhere, Petri dishes are unsuitable for the culture of algae. They should be replaced by flasks holding a much larger volume of the moist culture medium and furnished with narrow necks closed by very tightly rolled cotton plugs. At certain seasons it would be well to cover the plugs with the rubber caps made for that purpose.

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[2] 4 parts calcium nitrate, 1 part potassium nitrate, 1 part magnesium sulphate, 1 part potassium phosphate.

All dishes and solutions were carefully sterilized before the algae were sown in them. Instruments were sterilized immediately before use. In this way few if any organisms were added from outside when fresh cultures were made; but as, in order to save time, we started with small masses of material rather than with single cells or single filaments, our cultures were not pure. We experienced considerable annoyance in a few instances from the growth of bacteria or of algae other than the ones especially sought. Nevertheless, for such experiments as we had in mind, the extreme pains and the long time required to obtain pure cultures would hardly be justified by results. Furthermore, the behavior of living organisms in pure cultures may be due in part to two unnatural, that is to say unusual, factors, namely, the artificial culture medium and the vessel in which it is contained, and the freedom from the competition with other kinds of organisms and their products. Our aim was, therefore, to grow our algae under as nearly as possible natural conditions, though we realize that the degree of naturalness which we attained is only one stage in advance of the frank artificiality of pure culture.

One of the greatest difficulties encountered in the culture of green plants of any grade of complexity in a laboratory not provided with a greenhouse is in securing proper illumination, a difficulty all the greater when one realizes that not only is normal growth dependent upon adequate illumination, but many of the other phenomena of life are greatly influenced by it. As KLEBS (**8, 9**) and VÖCHTING (**19**) go far toward proving, the reproductive processes, as well as the behavior of the reproductive elements, are profoundly sensitive to light. Therefore, in any series of experiments involving irritability—that is to say, in every series of experiments on living organisms—the illumination must be carefully considered. Not having any greenhouses connected with our laboratory, our difficulties were increased. Furthermore, in our climate, there is a great contrast between the light of the rainy and of the bright days in winter, while in spring and summer, the direct sunlight is quite too intense for algae except in running water or in large bodies of still water. Between too little light at a short distance from a window and too much light near it, the right illumination is difficult to find. We attempted to attain this by placing our culture dishes on glass shelves in a window looking southeast, screening the dishes at times of bright sunshine by a sheet of white tissue paper pasted across the lower part of the window.

BEHAVIOR OF THE ZOOSPORES.

Locomotion.—While motile, the zoospores are evidently sensitive to light, moving toward the more strongly illuminated side of a slide, or coming to rest, when possible, on the light side of the dish in which they escape. This is a general statement of a result of our observation; but, as has been known since STRASBURGER'S classical study (**18**) of the behavior of swarmspores, they move toward light of a certain intensity rather than light of greater intensity, turning away from spots too intensely illuminated toward comparative darkness, just as they turn from comparatively dark spots to more suitably illuminated ones. Since there are still no means of accurately measuring either the intensity or the quantity of light falling upon a given spot, it is out of the question to determine what the minimal, optimal, and maximal illuminations are for motile zoospores, for their production, or for the parent plant. We must, therefore, content ourselves with reporting such general observations as we were able to make.

The duration of swarming has been reported by various authors (**15**, p. 768), among them STRASBURGER, who found active swarm spores of *Ulothrix zonata* after three days, of *Haematococcus lacustris* after two weeks, during which the material had been kept continuously in darkness. These figures have little significance beyond the fact that the plants produced motile zoospores during this time. The duration of swarming of the individual spores is quite unknown. On this point we can give little information. We found, however, that in our Oedogonium the zoospores came to rest and attached themselves within fifteen to eighteen hours, *i. e.*, zoospores which had escaped and were active in daylight came to rest and attached themselves during the succeeding night.

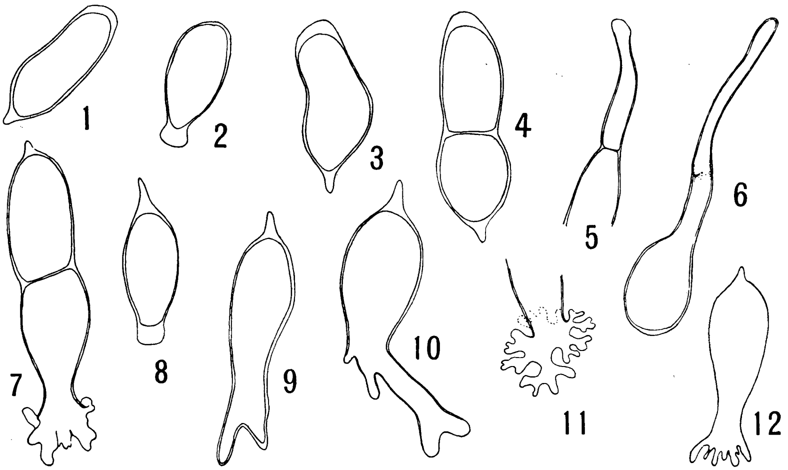
The spot to which zoospores formed in water attach themselves is largely determined by the direction and intensity of the light, if there be any, which falls upon the culture. If light of not too great intensity fall upon a dish horizontally or nearly so, the zoospores will collect and come to rest mainly upon the more strongly illuminated side. If on the other hand the light fall vertically or only somewhat obliquely from above, the zoospores will collect at the surface of the water. It is frequently observed, when the light falls obliquely upon a culture of algae, that the zoospores collect in greatest numbers at the surface of the water on the more brightly lighted side. From this the attractive influence of the oxygen of the air might be inferred. That such an inference is not always justified is proved by the fact that when the light falls horizontally, or is reflected obliquely from below upon a culture, the zoospores still tend to collect at the point best lighted, regardless of the greater oxygen supply near the surface of the water. As to the directive influence of oxygen upon the locomotion of zoospores, we made no experiments, but it is clear from this observation that in general the directive influence of light is stronger than that of oxygen. When, therefore, zoospores come to the surface of the water, they do so mainly under the influence of light. Their behavior on reaching the surface, which is decidedly different from that of the zoospores coming to rest elsewhere, we shall presently describe.

Germination.—When zoospores come to rest, the cilia are absorbed, the naked masses of protoplasm surround themselves by cell-walls, the forward end of each zoospore develops a holdfast attaching it to the surface with which it has come into contact. As light evidently directs the locomotion of the spore, it is reasonable to suppose that it also determines its polarity on germination. It is hardly probable that light influences the growth of the holdfast; nor has gravity any effect, for the spores attach themselves in various positions and may even be suspended from the surface of the water. Chemical stimulus can hardly have any part in the matter, except perhaps in relation to the bacteria. The growth of the holdfast would appear, then, to result from contact with a solid body, as an irritable response to contact stimulus. This matter we wish to report upon at length.

The influence of contact upon growing plants and their parts has been studied from various points of view by many investigators and upon a great variety of plants. It is now clear that contact with a solid object may influence growth in three ways: first, as to its direction, as shown by sensitive tendrils (**16**); second, as to its rate, also as shown by tendrils (**5**); and third, as to its kind, as shown by the formation of haustoria in dodder (**14**). Contact similarly influences the direction, rate, and kind of growth in germinating zoospores.

If it is true that contact influences the direction, rate, and kind of growth in germinating zoospores, the character of the surface with which the contact is effected should be reflected in the germinated zoospores, a rough surface producing a different and more pronounced effect than a smooth surface. To test this hypothesis, we used a variety of substances for the purpose of catching the zoospores, viz. wisps of cotton, clean cover glasses, glass roughened by corrosion and deposit, strips of gelatine, freshly split mica, and the surface of the water itself. These we interposed between the swarming spores and the light. The spores came to rest and attached themselves within eighteen hours.

Zoospores germinating on the surface of the water in contact with dust particles, diatoms, and masses of bacteria developed long slender processes, sometimes forked, but always hypha-like in appearance (*figs. 5, 6*). Others on the surface, and in contact with no solid matter whatever of visible amount, formed the most rudimentary hold-fasts, knob-like processes of very small size (*figs. 1, 2, 3, 4*). Those on gelatine, the acid reaction of which had been duly neutralized, formed knob-like or filamentous processes in lieu of holdfasts on clean spots on the gelatine (*fig. 8*), whereas those in contact with particles of dirt were more or less branched (*figs. 9, 10*). On roughened glass many zoospores developed disks (*fig. 12*). On clean glass the regularity of these disks was very striking (*fig. 11*). Irregularity of surface evidently induces irregularity of growth and form. This appears even more plainly from the experiments on marine algae (p. 343).



FIGS. 1–12.—Germinating zoospores of *Oedogonium sp.*? ×780.

1, 2, 3, Germinated on the surface of water (1, 2 filtered, 3 unfiltered) showing extremely rudimentary holdfast.—4. Same somewhat older, divided into two cells.—5, 6. Same but with hypha or rhizoid-like outgrowths at the basal end of each spore. Bacteria surround these filamentous processes.—7. Germinated on freshly split mica. Note well-developed holdfast.—8. Germinated on clean wet gelatine.—9. Germinated on a spot of dirt on wet gelatine.—10. Germinated on spots of dirt and bacteria on wet gelatine.—11. Germinated on clean cover glass. Note extremely regular holdfast.—12. Germinated on side of roughened glass dish. Note irregular holdfast.

Even clean glass and freshly split mica are not absolutely smooth. The dustless upper surface of clean water is smooth. So also is clean wet gelatine, as PFEFER's work on tendrils showed (**16**). On these two no holdfasts are formed by germinating zoospores. In place of holdfasts longer or shorter hypha-like filaments appear, often very short indeed, and branched only where the surface is roughened by dust or dirt. On carefully polished clean cover glasses, holdfasts of remarkably regular form were developed. As will be shown later (p. 344), uniformly ground cover glasses used in experiments with the less irritable germinating spores of marine algae induced the formation of very regular holdfasts. We may say then that the form, size, and even the development of holdfasts by sessile fresh-water algae depends upon the character, the degree of roughness, of the surface with which the zoospores come into contact. A uniform though slightly rough surface, like that of clean polished glass, will induce the formation of very symmetrical holdfasts. Coarsely or irregularly roughened surfaces induce the formation of irregular holdfasts. Extremely smooth surfaces fail to induce the formation of holdfasts, unless perhaps of the most rudimentary sort. It would seem clear then, that so far as the direction and kind of growth are concerned, contact acts as a stimulus; for the holdfast grows parallel with the surface, no matter how irregular this may be, and the organ produced corresponds to the surface on which it is formed, being simple or branched, symmetrical or irregular, according to the character of the surface. As to the effect of contact upon the rate of growth, we can say nothing so far as fresh-water algae are concerned. The measurements of growth rates among marine algae are given subsequently (p. 347).

From the observations thus reported, it is obvious that contact acts as a very important stimulus in the germination of the zoospores of sessile algae. The value of this to the plant is evident enough. If, however, we could induce algae ordinarily not sessile to form rhizoids or other holdfasts by bringing them into contact with suitably roughened surfaces, and if we could find, in nature,[3] algae ordinarily not attached fastening themselves to rough surfaces occasionally, we should add materially to the significance of our observations. Both of these things one of us has done. Though reserving the subject for further study, we may report now that plants of a species of Spirogyra which did not fruit and hence could not be determined while we were working upon them, formed rhizoids in our cultures, and were found to have formed rhizoids out of doors, wherever the filaments were in contact with sufficiently rough material, clean cover glasses, trough-scrapings containing lime, diatoms, etc., and similar

[3] Since this part of our work was ended, at least for the time, COLLINS'S paper in Rhodora (**6**:230. 1904) records the formation of holdfasts by Zygnema filaments growing against rock in a swiftly running stream.

material. We have never seen rhizoids on floating filaments or on the free floating parts of attached filaments. The size, form and direction of growth of the rhizoids were greatly influenced by the nature of the surface which the cells of Spirogyra touched, clean glass inducing the formation of strikingly regular crenate disks on the ends of the rhizoids like those formed on the same material by *Oedogonium*. Dirty glass, on the other hand, induced the formation of proportionally irregularly branching holdfasts. Thus our observations confirm the generally unknown observations of BORGE (3), who in 1894 reported his studies of a species of Spirogyra which formed rhizoids. BORGE'S paper contains a list of earlier authors who mention having seen rhizoid-bearing Spirogyra.[4]

From the fact that on extremely smooth surfaces like the surface film of clean water or of wet neutral gelatine, no true holdfasts form, and that on sufficiently rough surfaces even such a plant as Spirogyra may form organs of attachment, it is clear that the growth of rhizoids or holdfasts represents the reaction of a plant to the stimulus of contact. The germination of a zoospore of a sessile alga is influenced by contact; but contact, though it stimulate the zoospore to form an organ of attachment, is not needed to cause the zoospore to germinate. The zoospore may remain in motion for a long, but at present unknown, period of time. So long as it continues to move about, it does not germinate. When its locomotion is stopped, germination begins. When a zoospore reaches a very smooth but impenetrable sheet of gelatine interposed between it and the source of light falling upon a culture-vessel, it loses its cilia, ceases to move, and surrounds itself by a cellulose wall — it germinates; but unless it has stopped upon a particle of dirt on the surface of the gelatine, it puts out at best only a rudimentary hypha-like process, a rhizoid, not a holdfast, and this it does after germination has begun. Similarly, if the further progress of a zoospore toward the light be opposed by its having reached the top of the water in which it was found, the zoospore will cease to move, will lose its cilia, will surround itself with a cellulose wall, will germinate; but it will not form a holdfast or even a rhizoid unless it has happened to land upon particles of dirt or other solid. Germination, then, follows enforced cessation of locomotion; growth, so far at least as the formation of a holdfast is concerned, follows and is dependent upon contact irritation.

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[4] Subsequent papers by BORGE figure other species of Spirogyra with rhizoidal holdfasts (Die Algen der ersten Regnelschen Expedition. Archiv för Botanik 1:—— 1903).

Let us turn now to the marine algae.

II. MARINE ALGAE.

MATERIAL AND METHOD.

The material consisted of fresh fruiting plants brought in almost daily from the Bay of Naples. These plants, when not used immediately, were put into well-lighted aquaria, supplied with running sea-water, where they remained healthy for days after they had ceased to be used. The plants were *Dictyopteris* (*Halyseris*) *polypodioides*, *Dictyota dichotoma* (two varieties at least of this extremely variable form), *Cystoseira barbata*, *C. erica marina*, among brown algae; and *Laurentia obtusa*, *Polysiphonia* (sp. ?) among the red. I tried to get zoospores of Ulva, Cladophora, and other green algae, for the sake of comparison with the behavior of the zoospores of fresh-water algae; but whether owing to the season of the year (October, November) or to other causes, I do not know—at all events, I failed to get sufficient numbers to justify any conclusions. The spores used were of two sorts, the fertilized eggs of the two species of Cystoseira, and the non-sexual spores and tetraspores of Dictyopteris, Dictyota, Polysiphonia, and Laurentia. Their behavior is essentially similar.

BEHAVIOR OF THE SPORES.

Influence of light upon their escape.—I began my work with *Dictyopteris* (*Halyseris*) *polypodioides* and the two species of Cystoseira. Clean fruiting branches, as free as possible from diatoms and other organisms, were put in small dishes about 4^{cm} diameter and 2^{cm} depth, filled with fresh sea-water. To reduce evaporation the dishes were covered either with glass caps or clear glass plates. The dishes were set on a broad shelf inside a northwest window about 13^{cm} below the lower edge of the glass. The light fell therefore somewhat obliquely upon the cultures. At noon on sunny days I closed the screens, made of sheeting, in order to prevent the sun shining directly upon the dishes during the afternoon. I found this illumination satisfactory. If the fruiting branches are put in the dishes late in the afternoon, enough spores will escape by nine o'clock next morning to justify removing the branches. In this way the age of the escaped spores may be confined within fairly narrow limits. If it were desirable to limit the age of the spores still more, as was sometimes the case, I made the cultures as soon as the fresh material was brought in, in the forenoon, and left them for four hours on the window shelf. In this time many spores escaped from good material, and I removed the fruiting branches. The dishes were left otherwise undisturbed for 22 or 24 hours after the cultures were made, in order to allow as many spores as possible to stick closely by their slimy coverings to the bottoms of the glass dishes. At the expiration of this time, the water in the dishes was carefully decanted, and fresh sea-water poured in. A considerable number of spores may wash out in this first change, but presumably they are the youngest, the ones last escaped from the fruiting branches, and there is the advantage in losing them that the material left in the dishes is still more nearly of the same age. Within these 24 hours the spores of Dictyopteris will have begun to germinate, putting out a short blunt process which is to form the holdfast, and having divided into two or three cells. Cell-division, so far as the formation of a dividing wall is concerned, does not seem always to precede the appearance of the process which is to become the holdfast; but there seems to a be slight difference in this regard between spores germinating under the ordinary conditions of alternating daylight and darkness and those kept constantly in darkness. The latter generally form the division wall before putting out the process, but to this rule there are many exceptions. In Dictyota and Cystoseira, however, the division wall is always plainly visible before the rhizoid appears.

The escape of the spores of Dictyopteris is much less abundant if the dishes are left in the dark than if they are normally lighted. Thus, on November 11, at 12 o'clock, I put as nearly as possible equal quantities of fruiting Dictyopteris in eight dishes, two of which I set on the window shelf and six in the dark. At 10 o'clock the next morning I counted the spores in all the dishes. There were 444 and 75 spores in the dishes set on the window shelf. In the six dishes kept in the dark for these 22 hours there were 59, 134, 287, 135, 219, 218 spores. The first three of these dishes I then set on the window shelf, putting into each the same pieces of Dictyopteris that had been in them. The other three I set back in the dark, putting into them the same branches which they had previously contained. All the dishes had fresh sea-water put into them. At 5 o'clock that afternoon so many spores had come out in the dishes in the light that I removed the pieces of Dictyopteris from all six and left them undisturbed until November 14 in the morning. Then I counted the spores, practically if not quite all of which had become fastened to the bottom of the dishes. There were now in the dishes in the light 1565, 2046, and 2245 spores; in the dishes in the dark 866, 1716, and 596 spores. In the dishes put into the light after darkness there were now 5856 spores where there had been only 480 before, a gain of 5376 or 11.2 times as many. In the dishes kept in the dark there were now 3178 spores, a gain of 2606 or 4.5 times as many. Nearly two and a half times as many, therefore, had come out in the light as in darkness in the same length of time.

The counting of such large numbers of small spores, especially where they lie very close together, is not easy. To facilitate counting and to bring the count to an approach to accuracy, I ruled lines about 2.5^{mm} apart with India ink on the bottoms of the dishes. The counting was done over a white surface with a hand-lens magnifying about fourteen times.

To ascertain whether the amount of light has any effect on the discharge of spores, I put fruiting branches of Dictyopteris in dishes in the dark, in the full diffused light on the window shelf, and under black hoods which had a vertical slit on one side about 3^{mm} wide and 15^{mm} long. I did not count the spores, but it was evident that most had escaped in the dishes in full light, fewest in the dishes in the dark, and a quantity between these two in the diminished light.

Similar experiments with *Dictyota dichotoma* yielded results similar in every respect.

As indicating the relation of light to the process of extrusion of the gametes I may report some of my experiments on Cystoseira. On November 8, at 11 o'clock, I put fruiting branches of *Cystoseira barbata* in four small dishes, two of which I set in the dark, two on a shelf by a window, taking pains to have as nearly as possible equal numbers of fruiting tips in the different dishes. At the same time I similarly prepared four dishes of *Cystoseira erica marina*. In two hours and three quarters there were evidently many more spores (eggs) in the lighted dishes of *C. erica marina* than in those in the dark, but apparently about equal numbers in the two sets of dishes of *C. barbata*. At about 10 o'clock the next morning I removed the branches of *C. erica marina* from the dishes, throwing away those that had been kept in the light, and poured off the water from the four dishes. I counted 99 and 50 eggs in the two dishes which had been kept in the dark, 454 and 420 in the dishes kept in the light. The latter two had of course been dark for nearly or quite twelve hours, from sunset to sunrise. The branches which had been in the darkened dishes I put back in these dishes, with fresh sea-water, and set them on the window shelf. Four hours later many eggs had come out and I therefore removed the branches, but left the dishes quiet till the morning of the 10th, so that the eggs might settle and become fastened to the bottom of the dishes. On the morning of the 10th, I poured off the water, preparatory to counting the spores. Many spores were carried away by this means, but those attached to the bottoms of the dishes now numbered 270 and 241 respectively.

I made similar counts of the number of eggs of *C. barbata* escaped and attached in the dishes darkened for twenty-three and one-half hours. There were 426 and 348 in the two darkened dishes. There were so many spores in the dishes which had been exposed to the daylight till sunset and after sunrise that I contented myself with counting those in the first three spaces from one side of one dish. In this space there were 376 spores. This area equals about one quarter the area of the bottom of the dish. The spores were not equally distributed over the bottom, but there were at least four times as many over the whole area. There were therefore about four times as many spores in the dishes which had been in the daylight as in the constantly darkened dishes. As with *C. erica marina*, I put back the fruiting branches which had been in the darkened dishes, filled them with fresh sea-water, and set them on the shelf by the window. Three hours and a half later I took out the branches, leaving the spores which had escaped to settle in the dishes and to become attached. The next morning I counted the spores attached to the bottom of the dish, in which 426 spores had escaped and attached themselves in the dark. There were now 1574 spores. In three hours and a half, therefore, after putting the dishes in the light, 948 spores had escaped and become fastened to the bottom, more than twice as many as had escaped in twenty-three and one half hours of darkness.

That the number of spores which had escaped was far greater than the number which had attached themselves is shown by the other dish. From this dish I carefully decanted rather more than half the water and shook as many as possible of the loose spores into a space about 6^{mm} square. In this space a single layer of spores covered the whole area of $36^{sq\ mm}$. Half the area had two layers of spores, and one quarter had three layers. If these spores had been spread out in a single layer, they would have covered an area of $63^{sq\ mm}$. The average diameter of the spore is 0.09^{mm}. Calculating the area of a spore and dividing this into $63^{sq\ mm}$ we find that there were about 2623 loose spores in this area. There were many more loose spores than I could collect in one spot by shaking, so that the calculated number of practically spherical spores is none too large, though they could not occupy the whole of any quadrangular area. There must have escaped therefore at least 3400 spores during three hours and a half of daylight in the dish in which I had counted 348 spores, twenty-three and a half hours after the dish had been put in the dark. At least eight times as many spores had come out, therefore, in three and a half hours of daylight after darkness as in nearly seven times as long a period of darkness.

It is to be noted that this very large number of spores (egg-cells, probably fertilized) was obtained only after an abrupt change from protracted darkness to full daylight. In nature, except in polar regions, the darkness is never so prolonged; and nowhere in nature is the change from darkness to daylight so abrupt. Whatever effect darkness and light have upon the discharge of the gametes must be exhibited in maximum degree in such an experiment as I have just described.

We see, then, that light has a decided influence on the time and the rate of discharge of gametes (in Cystoseira) and of non-sexual spores (in Dictyopteris and Dictyota). In Fucus it has long been known that the majority of the gametes escape and fertilization takes place soon after sunrise. OLTMANNS (**12,** p. 523) speaks of a periodicity in the discharge of the gametes of the constantly submersed Fucaceæ (Halidrys, Cystoseira, etc.) as well as in Fucus. Fucus does not occur in the Bay of Naples, though abundant enough in most other parts of the ocean. It is ordinarily twice daily exposed at low tide, but as various authors[5] have shown such uncovering is not necessary to the discharge of the gametes. The mechanism of the discharge of the gametes consists, in part, in the swelling of the gelatinous material in the conceptacles and the compression of the walls of the conceptacles by the surrounding tissues when the sexual elements are ripe. OLTMANNS expresses doubt whether these two factors alone furnish an adequate explanation of the process. As the matter now stands, these mechanical means do not account for the periodicity, unless we assume the relation of light to the processes connected with the growth of the gametes and with the formation of gelatinous matters within the conceptacles.

The periodicity coincides with the sequence of light after darkness, and is as evident, as my experiments have shown, in forms having no conceptacles as in those that do have them. In Dictyopteris, the aplanospores (tetraspores) form on both surfaces of the flat thallus, near the midrib, and project more or less from the surfaces as they grow. Finally they escape through a slit in the wall of the mother-cell. They may even germinate (**12,** p. 486) before escaping. It is difficult to see where mechanical pressure can effectively develop here except in the sporangium itself, that is, by the growth of the spores and the gelatinization of the wall and residual contents (if any) of the spore mother-cell. I shall subsequently show that light favors the germination of the spores and the growth of the young plant, though they will germinate and grow in darkness. It is probable, therefore, that light favors the growth of the spores before they escape from the sporangium as well as after. In this we have a partial explanation of the connection of the periodic discharge of the spores in Dictyopteris and of the gametes in Fucus and Cystoseira with the mechanical means of discharge above discussed. The influence of light is favorable to the growth of the spores, therefore to the development of mechanical pressure by them, but as the rapid discharge of spores occurs within a few hours after the fruiting material has been put into the light after being in darkness, it may be objected that there is not time for sufficient growth to take place to develop the necessary mechanical pressure, if mechanical pressure has anything to do with the matter in such plants as Dictyopteris. In reply to this possible objection, I may say that, though the spores are rapidly discharged within a few hours, there was no evident increase in the accumulation of spores in my dishes until at least two hours had elapsed after they were put in the light. There is therefore time for growth.

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[5] Cited by OLTMANNS, *l. c.*

To make this point clear I will report one experiment. Three small dishes of fresh fruiting Dictyopteris branches were kept in the dark for twenty-two hours. Early in the morning, the material was removed from these dishes, in which a considerable number of spores had been discharged, and placed in three clean dishes of fresh sea-water on the window shelf in the light. From these dishes the fruiting branches were removed after the lapse of one hour and put in another set of three dishes, the first set being left otherwise undisturbed to allow the spores which had escaped to settle and become attached. The same was done at the end of each of the two hours following. At the end of another hour and forty minutes, I repeated the process, and again after the lapse of two hours more. I therefore had five sets of three dishes each, in which spores had escaped. The number of spores at the time of exposure to light are as follows:

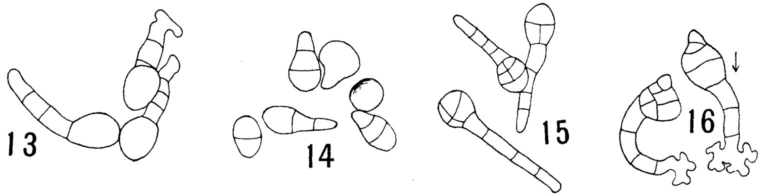
After 1 hour's exposure to light	36	spores in	3	dishes
" 2 hours' " "	26	"	3	"
" 3 " " "	170	"	3	"
" 4⅔ " " "	1671	"	3	"
" 6⅔ " " "	597	"	3	"
Total number of spores in 15 dishes	2500			
Total number of spores in escaped last 3⅔ hours . .	2268			
Percentage of spores escaped in last 3⅔ hours . . .	90			
Percentage total time in which these spores escaped .	55			

From these figures it is clear that the discharge of the spores in the light is not an immediate one, but that a certain length of time must elapse after the plants are brought into light after darkness before they begin to discharge their spores rapidly. What changes take place under illumination which result in the discharge of the spores, I do not venture even to guess. On this interesting point I have no data.

It may be that in addition to the influence of light on the processes of food manufacture and growth in the spores and in cells adjacent to the sporangia, there are irritable responses which contribute to the development of the mechanical pressure which causes the spores to be discharged. Information on this point might add to our meager knowledge of the phenomena of irritability in the marine algae.

I am by no means satisfied that the plant does not irritably respond to the light by more immediate means than growth. The light may also favor gelatinization, but the connection with this process, the details of which are unknown, is not sufficiently clear to justify discussion now.

The influence of light upon the germination of the spores.—The germination of the spores of Dictyopteris has been adequately described by REINKE (**17**), but he naturally made no attempt to ascertain the influence of the different factors of the environment upon the course of germination and upon the form of the young plants. In order to learn something about these matters I compared spores which had escaped during twenty-four hours in different dishes as to their rate of germination and the development of the young plants in the light and in darkness. In *fig. 13* we have young plants shown which had developed thus far in a dish completely covered by black paper except for a vertical slit 17×6^{mm} on the side of the dish toward the window. The time since sowing was three and three-quarters days. In *fig. 14* are shown young plants from spores escaped at the same time, but in dishes kept completely darkened. It may be objected that the conditions of the two experiments were not the same, and therefore the results may not be comparable. I have shown above that light favors the escape of the spores, probably because it favors their growth. For this reason, therefore, the results are exactly comparable, because the point is made still clearer that the germination of the spores is more rapid under the normal daily alternation of light and darkness than in continuous darkness. For the germination of the spores of ferns (**4**, p. 423–4), the seeds of Viscum, and for various other plants it is claimed that light is necessary. This is not generally the case with higher plants. As *fig. 15* shows, the young plants grown in the dark for five days were only as far along as those grown for three and three-quarters days under the black cap with a slit in the side. *Fig. 16* shows plants five days old grown under the black hoods with a side slit. This difference in growth rate may be due to two factors at least. In the first place, no manufacture of non-nitrogenous food can take place in the dark, though some such food may be manufactured even in the dim light under the black cover. If such food were manufactured, it could be used at once to nourish the organism, which is supplied in the spore with only a comparatively small amount of food. In the second place, if the manufacture and assimilation of nitrogenous food is more rapid in the light than in darkness, as is now claimed,[6] those spores in the light would be better off than those in darkness.



FIGS. 13–16.—*Dictyopteris* (*Halyseris*) *polypodioides*. ×123.

13. Germinating spores sowed 3¾ days earlier on ground glass in dish receiving light only through vertical slit on one side, $17 \times 6^{\mathrm{mm}}$; direction of light indicated by arrow. Note that all rhizoids point away from light, whereas the nearly erect plantlets are inclined toward the light.—14. Same in every respect, except that it was kept constantly in darkness. Note smaller size of plantlets. 15. Same, five days old, constantly in dark. Size about same as lighted plantlets 3¾ days old.—16. Same as 15 but in dish lighted through vertical slit, $17 \times 6^{\mathrm{mm}}$. Plantlets strikingly further advanced, erect but inclined toward light; direction of source of light indicated by arrow.

Whatever the reason may be, the young plants grow and develop faster in normally alternating light and darkness than in continuous darkness. This fact, not altogether in harmony with certain current views of the influence of light and darkness upon growth (**11**), deserves much more extended study. It seems to us probable that the opinions of plant physiologists, based too exclusively on studies of land plants, will be modified when the algae, especially the marine algae, have become as familiar to them as aquatic animals now are to other physiologists.

Having seen that light favors germination and growth, we may now inquire what other effects it has. If spores sown in dishes in the manner previously described are exposed to one-sided illumination, the holdfasts will appear always on the side away from the light. If spores germinate in darkness, the holdfast will be put out in all possible directions from different spores. This result I have repeatedly obtained in Dictyota, Dictyopteris, and Laurentia, as well as in two species of Cystoseira, thus confirming and extending WINKLER'S (20) interesting experiments on *C. barbata.* The gelatine method which WINKLER used for part of his experiments is apparently more exact than mine, but what it gains in apparent exactness it loses in naturalness. The spores in a layer of sea-water not too thin (as it may be on a slide, especially if this is covered) quickly adhere to the surface of clean smooth glass, and if the dish remain undisturbed for several hours they are not likely to be dislodged by ordinary movements afterwards. The use of gelatine, or of any other similar material even in dilute solution, such as WINKLER describes, is open to suspicion by reason of the facts reported in the foregoing part on fresh water algae, and also because of the results of BORGE (3) already referred to, although WINKLER reports the germination as perfectly normal. Naturally, for his gravitation experiments, a solid medium was necessary, but not for those on light.

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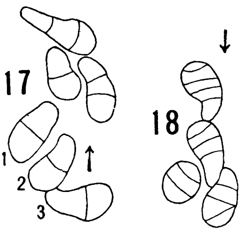
[6] GODLEWSKI's papers in Bull. Acad. Sciences Cracow, 1903, and earlier and later.

To escape the legitimate objection that the spores may behave differently in darkness and in light, I put upon the horizontal plate of a clinostat a dish in which Dictyopteris spores had escaped during twenty-four hours in darkness, covering the dish with a black sheet of cardboard so that all the light came from the side. The plate of the clinostat was also covered with dull black paper to avoid possible reflection from below. The dish was therefore revolved in a horizontal plane and received light not only from the side, but on all sides successively. The clinostat was very simple — an alarm clock from which face and hands were removed, a sleeve carrying a thin zinc plate being soldered to the minute hand spindle. The dish was therefore revolved once an hour. The result was that the germinating spores sent out holdfasts in all possible directions, some of the rhizoids growing upwards, others downwards, the majority horizontal until their increasing weight tipped the spores sufficiently to bring the ends of the rhizoids into contact with the glass.

It appears, then, that the side of the spore which is to give rise to the holdfast is ordinarily determined by light, which in nature falls from above, vertically or more less obliquely. The direction of growth of the rhizoid is also determined by the light, other things being equal, and this frequently brings it into a more or less vertical position until it comes into contact with a solid object.

Although under natural conditions the cell which is to form a rhizoid, and the direction of growth of the rhizoid, appear to be determined by light and by the direction from which it comes, the fact that spores will divide and form rhizoids both in the dark and under supposedly equal illumination from all sides successively, leaves us with a doubt whether, after all, the point of formation of the rhizoid may not depend on other factors also. Keeping the spores continuously in the dark for forty-eight hours or more is extremely unnatural. The results of such procedure may also be unnatural; they may mislead us as to the natural course of events. Again, cultivating the spores in a dish on a clinostat is an unnatural proceeding, for in nature the illumination is not even approximately equal on all sides in one plane, but is unequal and in many planes successively. And finally, though darkness follows light and light follows darkness only gradually, on the clinostat as well as in nature, it is obvious that a spore in a dish on the clinostat receives the stimulus of light on one side, and as darkness comes on, there can be only weaker and weaker stimuli, and finally none at all on the other sides of the spore. The direction of division of the spore may or may not be determined by this last stimulus of light. According to WINKLER (**20**, p. 302), a three or four-hour exposure to one-sided illumination is necessary to determine the direction of the first division wall in the eggs of Cystoseira. He suggests that perhaps by changing the direction of illumination 90° or 180° about every three hours, we might stop the germination altogether. This I have not tried with Cystoseira, but as the foregoing shows, such is not the case with Dictyopteris spores, for they germinate in darkness, and also on the clinostat, at once, although perhaps not as rapidly as under ordinary illumination. It may take three or four hours under ordinary conditions to determine the line of division of an egg or spore. It may take much less time, much less of a stimulus by light, to determine which of the two cells thus formed shall send out the rhizoid. In cultures of *Dictyota dichotoma* under one-sided illumination, but with the ordinary alternation of daylight and darkness, I noticed that both the direction of divisions that took place at night and also the cell which put out the rhizoid did not conform to the rule to which those spores which germinated in the daytime evidently pointed. This is clear from *figs. 17* and *18*. Rhizoids, formed during the night on the side which did and will receive more light during the day, bendaway from the light when the daylight comes. In such cultures as these we have much more nearly normal conditions than in continuous darkness or on the clinostat. Divisions often occur and the rhizoids often grow out in darkness, and though the spores were lighted only from one side during the day, the direction of these divisions and the side from which the rhizoid springs follow no rule. This fact at least suggests, but without proof, that influences preceding illumination also affect the direction of division of the spores, and the point of origin of the rhizoids.



FIGS. 17, 18.—*Dictyota dichotoma*. ×123.

17. Germinating spores 1¾ days since sowing on ground glass in dish lighted only through vertical slit, direction of light indicated by arrow. Note that spores 1, 2, 3 divided during the night (in darkness), and that the rhizoids of 2 and 3 are curving away from the light.—18. Somewhat older.

It does not seem to me necessary to assume a polarity in the spore. There is no evidence in the form of the undivided spore that there is such a polarity, for the undivided spore is spherical or spheroidal. After the first division the spore becomes ellipsoidal by the growth of the daughter cells at right angles to the division wall. The rhizoids grow from one or the other end—sometimes from both ends—of these ellipses, not from the side; but beyond this there is no indication of the point of origin of the rhizoid. So far as I can see, then, all one is justified in saying is that, in the light, the direction of division of the spore and the point of origin of the rhizoid are determined mainly by the direction from which the light falls upon them, but that the spore has the impulse both to divide and to form a rhizoid, and will do both even in the absence of any directive influence. In the latter case the direction of division and the point of origin of the rhizoid are determined by conditions preceding the escape of the spore from the mother cells, conditions which may have induced a polarity in the spore, but a polarity which is not so fixed that subsequent influences cannot alter it.

Influence of contact on germinating spores.—From the work in fresh-water algae reported in the foregoing pages, one is led to suspect that the character of the surface with which the rhizoids of germinating spores of marine algae come into contact will largely determine the shape and completeness of attachment of the holdfasts. The strength of the holdfasts as the plants mature is determined not merely by the surface but by other factors. Among these may be mentioned the movement of the water in currents and waves, the size and shape of the plants, and all other factors which affect the strain which the holdfast must withstand.[7] But the first attachment of sessile algae, whether marine or fresh water, is greatly influenced by the roughness of surface. I was first led to suspect this by the extraordinary freedom of Spirogyra and Iridea—the smoothest, most slippery fresh and salt water algae, respectively, which I know—from diatoms and other sessile plants growing upon them. Cladophora and Microcladia, comparatively rough forms, show the direct opposite.

To test this hypothesis with marine algae, I put in the bottoms of the glass dishes used for cultures cover glasses 18×18^{mm} square, which had been carefully ground on one side on a stone. One half of the cover glasses were put smooth side up, the other half rough side up. At first I used the cover glasses only rough side up, letting the smooth glass of the dishes furnish the other surface. This plan seemed open to objection, as the glass of the dishes might not have the same composition as the material of the cover glasses, and the light would not be the same on a ground surface as on a smooth. This latter objection is not wholly eliminated even by using thin cover glasses, but it is as nearly eliminated as at present possible.[8] The cover glasses ground on one side only, some with the ground surface upward, others with the ground surface downward, served as the bottom on which the spores settled.

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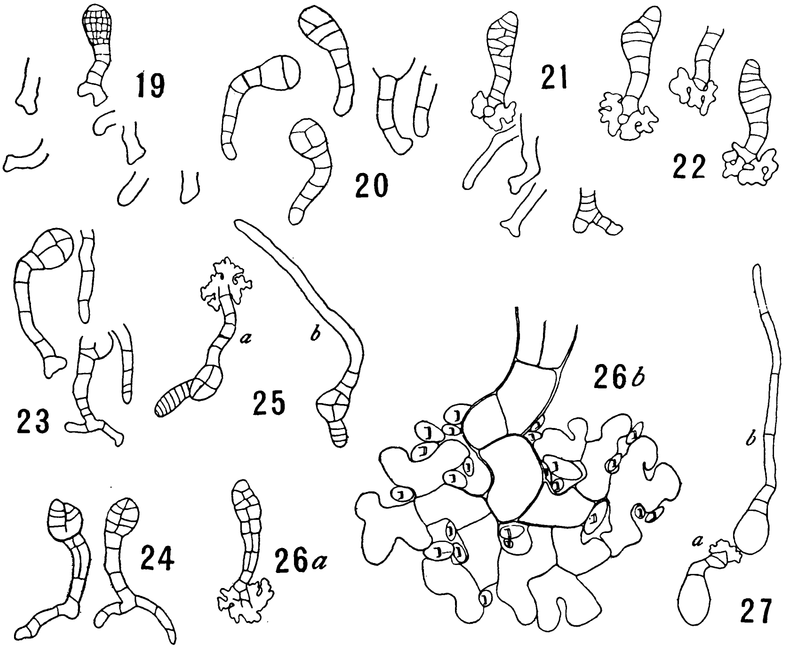
[7] PFEFFER (1, p. 305) has felt obliged to retract his published conclusions, based on the work done in his laboratory by the late R. HEGLER (6), that an increased strain acts as a stimulus to the development of increased strength, as shown in part by anatomical changes in the tissues; but I may venture to express the belief that time will show that PFEFFER and HEGLER and not PFEFFER and BALL (1) were right.

[8] The objection can be and was eliminated in certain cultures by keeping them in the dark. The results were similar to those in the light, but darkness necessarily introduces other effects if prolonged.

In these experiments I used the same species previously enumerated, but those which form a single rhizoid at first give more immediately recognizable results than those which, like Cystoseira, form several rhizoids. For this reason Dictyota, Dictyopteris, and Laurentia are preferable. I shall describe first Dictyopteris, as it was the marine alga with which I first obtained satisfactory results. The results obtained with Laurentia are so similar that I shall not speak of them further.

In *fig. 19* we have sketches of Dictyopteris plantlets two and five-sixths days after sowing on ground glass. *Fig. 20* shows plantlets sowed at the same time on smooth glass. In the first set the rhizoids are beginning to enlarge, and one to branch, at the tips. In the latter there is no such appearance. The idea that this difference in appearance might be due to a difference in age suggests itself. But the difference in age could be only slight, for the fertile branches were removed at the same time from both dishes, and both dishes were emptied and filled with fresh sea water at the same time. Emptying the dishes generally removes the youngest spores, those still unattached or only slightly so, leaving those which are equally firmly attached. I am nevertheless inclined to think that not merely is growth in the part of the rhizoid in contact with a rough surface stimulated by that contact, but also the growth and development of the other parts of the plantlet also. There seems to be a transfer from cell to cell of the stimulus produced by contact, a stimulus the greater the rougher the surface. If this be true, there is every reason for the younger appearance of the plantlets on the smooth glass, for their growth was less stimulated than that of the other plantlets. This is in accord with the observations previously referred to (**10**) which Loeb made upon animals.

Fig. 21 shows the same group of plantlets nearly twenty hours later. Here holdfasts are evidently begun. Twenty hours later than this we have the condition shown in *fig. 22*, where the rhizoids have almost completely lost their filamentous appearance and have become holdfasts, circular in general outline, but with elaborately crenated margins. Comparing these two figures with *figs. 23, 24,* showing plantlets of the same age on smooth glass, we see that the plantlets have not changed in character from day to day, though they have grown; that the rhizoids are still filamentous; and that no holdfasts are yet begun.



FIGS. 19–26.—*Dictyopteris polypodioides.* ×123, except 26 *b*, ×900.

19. Germinating spores 2⅚ days since sowing on ground glass.—20. Same in every respect except sown on unground glass. Note that plantlets appear younger than those of same age on ground glass.—21. Same as *fig. 19*, but 3¾ days since sowing.—22. Same as *fig. 21*, but 24 hours later, 4¾ days since sowing.—23. Same as *fig. 20*, 3¾ days since sowing.—24. Same as *fig. 23*, 24 hours later, 4¾ days since sowing.—25. Ten days since sowing, *a* on ground side of cover glass, *b* on unground side, in sea water.—26*a*. On unground cover glass.—26*b*. Same showing diatoms and diatom-shells, *D*, in almost every lobe of the well-developed holdfast.

FIG. 27.—Two *Dictyota* plantlets on smooth glass. ×123.

a has formed a holdfast on coming into contact with dirt; *b* finding no such obstacle has grown out into a long rhizoid; *a* erect, *b* erect at tip but rhizoid creeping.

In *fig. 25* we see the condition of two plantlets of Dictyopteris ten days after sowing, the one on ground glass, the other on smooth. These are not the extremes of two series of plants, but are the average. On unground glass, however, under certain conditions, as elaborate holdfasts may develop as on ground glass. When I saw the plant shown in *fig. 26a*, I thought my hypothesis was disproved, for here we have as elaborate a holdfast as on ground glass. On examining the plant with a stronger magnification, I found, as *fig. 26b* shows, that diatoms or diatom-shells were very numerous on the glass at this point, making it rough, or rather covering it with obstructions to the growth of the base of the plantlet. This plant and the many others like it confirm my belief that roughness of surface stimulates the sessile algae firmly to attach themselves.

It must be noted, however, that ground cover glasses kept in cultures for a week or so become slimy; their roughness is reduced or concealed by a thin, very smooth coat, which must be dissolved off before one can use the cover glasses again with the maximum effect. Even when all "dirt" and diatoms are washed off, the cover glasses are not clean as they are when new; they must have the slimy coat removed too. This is easily done by boiling in fresh water for a few moments. On the other hand, diatoms, animal excrement, and fine dirt generally, often so roughen the unground surface of glass as to induce holdfast formation of marked character.

When for any reason, such as the extreme smoothness of the glass, or the absence of any contact whatever, the elongation of the rhizoids is not stopped, they may attain surprising lengths and often in a very short time. *Fig. 25* shows such an extraordinarily long rhizoid of Dictyopteris. *Fig. 27* shows us two plantlets of *Dictyota dichotoma* growing on the same smooth side of a cover glass. In the one case, the rhizoid soon came into contact with small particles of dirt sticking to the glass, and thereupon ceasing to grow in length, formed a typical holdfast. In the other case, no dirt caught the rhizoid, which continued to grow over the smooth slime-covered glass.

I may here say something about the amount and the rate of growth of these germinating spores. On November 5th, at a quarter past twelve, I made a camera drawing of a spore of *Dictyota dichotoma.* Four hours later I similarly drew the same spore. And the next morning at a quarter to eleven o'clock I made another drawing. This third drawing is 107 per cent. longer than the first and 85 per cent. longer than the second. Another spore was 0.089^{mm} long when the first drawing was made. In four hours it grew 0.016^{mm}; in twenty-two hours and a half it grew 0.089^{mm}; and in forty-five hours and a quarter it grew 0.2195^{mm}. These figures converted into rates of growth per hour show that the plantlet increased in length at the rate of 4.23 per cent. per hour for the first four hours, 4.4 per cent. per hour for the next eighteen hours, and 5.82 per cent. per hour for the following twenty-three hours. These figures, however, distribute the growth over the whole length of the young plant, whereas it is mostly at the tip. Since this is the case, the rate of growth in the growing part must be much higher. I was not able at the time to make measurements to ascertain this rate in Dictyota. Similarly a spore of *Dictyopteris polypodioides* increased in length 360 per cent. in three days, or an actual increase in length of 0.292^{mm}.

All of these cases of growth are unusual. The plantlets sent out their rhizoids either horizontally or somewhat obliquely upwards, at all events without contact with the surface of the glass. Where contact occurs, there is no such extraordinary growth unless the contact be with an exceedingly smooth slippery object. There, as in water, the rhizoid will continue to elongate.

In a spore of Dictyopteris I attempted to measure the length of the growing region, the total increase in length, and the distribution of this increase among the cells of the rhizoid; but the growth rate in this instance has little value, because of the extraordinarily cold weather prevailing at the time. As the laboratory is cold at night, the growth rate for twelve hours of the twenty-four must have been much below normal. However, I will give the figures. The increase in length of the tip cell of the rhizoid was 150 per cent. or 0.078^{mm} in twenty-four hours. The increase in length of the cell next behind was 40 per cent. or 0.020^{mm}. The other cells of the rhizoid did not lengthen at all. Of the total increase in length 79 per cent. was made by the tip cell. This cell had at the outset only 41 per cent. of the length of the growing region. The actual zone of growth, however, is only the tip of the cell. Hence these percentages give only a vague and quite inadequate idea of how rapid the growth is in the actually growing part.

SUMMARY.

1. The zoospores of Oedogonium, as has long been known, are sensitive to light, the direction of locomotion and the place at which they come to rest being determined much more by the direction and

the intensity of the light falling upon a body of water than by other influences, such as unequal distribution of oxygen, etc.

2. Apparently the germination of the zoospores of sessile algae is induced primarily by interference with their locomotion. When this is blocked, germination begins; conversely, when nothing prevents locomotion, they do not germinate.

3. The nature of the attachment formed by the germinating zoospores of sessile algae is dependent upon the roughness of the surface of the object with which they come into contact. Upon extremely smooth surfaces—such as the surface of clean water and clean wet gelatine—the spores form either only the shortest most rudimentary holdfasts or merely rhizoids; whereas, on relatively rough surfaces, the holdfasts are large and conform in their lobing to the contour of the surface. Furthermore, ordinarily floating algae may sometimes be induced to form rhizoids or other organs of attachment if brought into contact with sufficiently rough surfaces.

4. The discharge of the spores or gametes of Dictyopteris, Dictyota, and Cystoseira is strongly influenced by light, the discharge being much more rapid within a few hours after exposure to light than before or than in continuous darkness. For this reason the time of the discharge as well as the rate is strongly influenced by light, and a periodicity of discharge is established which follows approximately the periodicity of daylight and darkness.

5. The spores of the sessile marine algae studied germinate better in normally alternating daylight and darkness than in continuous darkness; and subsequent growth and development follow the same rule.

6. As shown by WINKLER to be the case with *Cystoseira barbata*, we have found that the direction in which the light falls determines the plane of the first division in the germinating spores of *Cystoseira erica marina*, Dictyopteris, and of Dictyota, the new cell wall being formed at right angles to the incident rays.

7. Similarly, the rhizoids or holdfasts formed by germinating spores ordinarily issue from the daughter cells away from the light. In darkness the rhizoids arise in all possible directions, sometimes even from both cells of a germinating spore.

8. The direction of growth of rhizoid and of plantlet is determined

mainly by the direction from which the light comes: the rhizoids are negatively phototropic, the plants positively.

9. As in fresh-water algae, so also in sessile marine algae, the nature of the surface with which the spores come into contact very largely controls the nature of the attachment formed, a rough surface inducing the growth of a large and well-developed holdfast, a smooth surface causing proportionately less growth.

10. Though the direction toward which the rhizoids ordinarily grow is determined at first by light, the character of the surface with which the rhizoid comes into contact still more strongly influences the direction of its growth.

11. The direction, rate, and kind of growth of these germinating spores is strongly influenced by contact irritation.

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